^{1}H NMR spectrum was recorded in $CDCl_3$ and the data presented in the text. Periodic acid consumption of lanceolarin alongside adicardin and followed by the spectrophotometric method of Aspinall and Ferrier [5] revealed the uptake of 3.80 and 3.98 mol, respectively of periodic acid. Details of permethylation of lanceolarin and adicardin and their hydrolytic studies are already reported in connection with the structural studies on adicardin [3].

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ABSOLUTE CONFIGURATION OF (+)-5,6-DEHYDROLUPANINE, A KEY INTERMEDIATE IN BIOSYNTHESIS OF LUPIN ALKALOIDS

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Key Word Index—*Thermopsis chinensis*; Leguminosae; aerial parts; lupin alkaloid; quinolizidine alkaloid; (+)-5,6-dehydrolupanine; lupanine; absolute configuration; biosynthesis.

Abstract—(+)-5,6-Dehydrolupanine, a key intermediate in biosynthesis of lupin alkaloids, was isolated from *Thermopsis chinensis*. The absolute configuration of the compound was determined to be 7*R*,9*R*,11*R* by chemical transformation to (−)-lupanine.

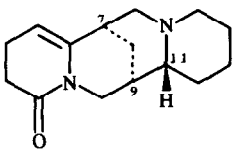
INTRODUCTION

(+)-5,6-Dehydrolupanine (**1**) is rather widely distributed in the Leguminosae, although usually as a minor alkaloid [1]. Compound **1** has been postulated as a key biosynthetic intermediate between the sparteine-type alkaloids, e.g. lupanine, and the α-pyridone-type bases, e.g. anagyrine [2–4]. However, the absolute configuration of **1** has not been clarified. In the present investigation, we have determined the absolute configuration of (+)-5,6-dehydrolupanine (**1**) from *Thermopsis chinensis* as 7*R*,9*R*,11*R* by chemical transformation of **1** to (−)-lupanine (6*S*,7*R*,9*R*,11*R*) (**2**).

RESULTS AND DISCUSSION

From the 75%-EtOH extract of the aerial parts of *T. chinensis*, **1** was isolated in a yield of 0.002% of the fresh weight by repeated chromatography. We also isolated seven known lupin alkaloids, (−)-anagyrine (main base), (−)-*N*-methylcytisine, (−)-baptifoline, (−)-cytisine, (+)-lupanine, (−)-*N*-formylcytisine and rhombifoline [5].

The relative stereostructure of **1** was identified by the analysis of ^{13}C and ^{1}H NMR, mass spectrometry, IR and UV data and comparison with those reported previously [3–22]. In its CD spectrum, **1** showed a negative Cotton



(7*R*:9*R*:11*R*)

1

effect at 310 nm ($[\Theta]_{310}$ −930) and positive effects at 258 nm ($[\Theta]_{258}$ +8000) and at 234 nm ($[\Theta]_{234}$ +11 000. These are the same Cotton effects as those of (−)-anagyrine ($[\Theta]_{310}$ −13 000, $[\Theta]_{233}$ +17 000) and (−)-cytisine ($[\Theta]_{310}$ −21 000, $[\Theta]_{233}$ +43 000). These results suggested that the absolute configuration of **1** is 7*R*, 9*R*,11*R*, the same as that of (−)-anagyrine and (−)-cytisine.

The final confirmation of the absolute configuration of **1** was made by chemical transformation of **1** to (−)-lupanine (**2**) by catalytic hydrogenation on platinum dioxide. The absolute structure of **2** was identified by $[\alpha]_D$, CD, IR, ^{1}H NMR, mass spectrometry, GC and HPLC. These results clearly indicate that **1** has the same absolute configuration as that of **2** (7*R*,9*R*,11*R*).

Compound **1** has been assumed to be an important key intermediate in the biosynthetic pathway of tetracyclic lupin alkaloids between sparteine and α-pyridone type bases [2–4]. Several papers have reported the presence of **1** in many species in which both sparteine and α-pyridone alkaloids are detected [6–22]. Nevertheless, the absolute structure of **1** has never been clarified, presumably because of the lack of sufficient weight of the material. The present study has clarified that the absolute configuration of **1** is the same as those of (−)-lupanine [23] and (−)-anagyrine [24]. These findings make it more plausible that **1** is the biosynthetic intermediate from (−)-lupanine to (−)-anagyrine. Recently, Asres *et al.* [17] have identified an unstable alkaloid, 6β-hydroxylupanine, and proposed that this compound might be a biosynthetic intermediate between lupanine (**2**) and 5,6-dehydrolupanine (**1**). It would be interesting to determine the absolute configuration of 6β-hydroxylupanine in order to make the biosynthetic linkage between these alkaloids more certain.

EXPERIMENTAL

Extraction and isolation of alkaloids. Aerial parts (leaves and stems) were harvested in July at the Medicinal Plant Gardens, Chiba University. The total alkaloid fr. (1.9 g) was prepd from 1.2 kg of fr material as described previously [25]. This fr. was chromatographed repeatedly by silica gel CC with CH_2Cl_2–MeOH–28% NH_4OH (500:5:1) to give the **1**-rich fr containing a small amount of (−)-anagyrine. Pure **1** (25 mg) was obtained from this enriched fr. by silica gel CC with CH_2Cl_2–MeOH–28% NH_4OH (1000:3:1).

(+)-*5,6-Dehydrolupanine.* Oil, $[\alpha]_D$ +37.3° (EtOH; *c* 0.25). CD (MeOH) $[\Theta]_{310}$ −930, $[\Theta]_{258}$ +8000, $[\Theta]_{234}$ +11 000. EIMS (probe, 70 eV) *m/z* (rel. int.): 246.1731 $[M]^+$ (37) ($C_{15}H_{22}N_2O$, calc. 246.1731), 163 (7), 148 (6), 136 (14), 135 (10), 134 (11), 98 (100), 97 (37), 69 (50), 41 (25). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2930, 2850, 2800 (Bohlmann bands), 1650 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ4.90 (1H, *dd*, *J* = 5.6 and 3.4 Hz, H-5), 3.98 (1H, *d*, *J* = 13.4 Hz, H-10_{eq}). ^{13}C NMR (67.8 MHz, $CDCl_3$): 19.2 (*t*, C-4), 21.5 (*t*, C-13), 22.8 (*t*, C-14), 25.2 (*t*, C-8), 27.4 (*t*, C-12), 31.8 (*t*, C-3), 33.2 (*d*, C-9), 34.2 (*d*, C-7), 48.1 (*t*, C-10), 54.7 (*t*, C-17), 56.5 (*t*, C-15), 63.3 (*d*, C-11), 102.3 (*d*, C-5), 143.0 (*s*, C-6), 170.9 (*s*, C-2).

Catalytic hydrogenation of **1**. **1** (20 mg) was dissolved in 5 ml of HOAc and hydrogenated at 1 atm over PtO_2 at room temp. for 30 min. The resulting soln was filtered and concd *in vacuo*. The product was purified by silica gel CC (1.6 × 7 cm) with CH_2Cl_2–MeOH–28% NH_4OH (1000:2:1) to give 10 mg of pure **2**, $[\alpha]_D$ −55.2° (EtOH; *c* 0.1), CD (MeOH) $[\Theta]_{223}$ −10 000. EIMS (probe, 70 eV) *m/z* (rel. int.): 248.1886 $[M]^+$ (58) ($C_{15}H_{24}N_2O$, calc. 248.1886), 136 (100), 98 (53). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2980, 2930, 2850 (Bohlmann bands), 1620 (C=O). ^{1}H NMR (400 MHz, C_6D_6): δ4.76 (1H, *dt*, *J* = 13.1 and 2.5 Hz, H-10_{eq}), 2.71 (1H, *m*, H-6), 2.63 (1H, *dm*, *J* = 11.9 Hz, H-15_{eq}), 2.53 (1H, *t*, *J* = 11.3 Hz, H-17_{eq}), 2.26 (1H, *dd*, *J* = 13.1 and 2.7 Hz, H-10_{ax}). **2** was also identified by GC (OV-17, 2 m, 250°) and HPLC (Lichrosorb Si-60, 25% MeOH in Et_2O–5% NH_4OH (25:1).

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